DATA EVALUATION RECORD

CAPHRA/TRANSPERMETHRIN

Study Type: OCSPP Non-Guideline; *In Vitro* Metabolism Kinetics

EPA Contract No. EP-W-16-018
Task Assignment No.: 32-3-013 (MRID 50600306)

Prepared for
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STUDY TYPE: OCSPP Non-guideline; *In Vitro* Metabolism Kinetics.

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TEST MATERIAL (PURITY): Transpermethrin technical (98.5%)

SYNONYMS: (*S*)-cyano(3-phenoxyphenyl)methyl (1*R*,3*R*)-3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropanecarboxylate

CITATION: Brown, S. (2018) Transpermethrin: a study to determine the kinetics of metabolism of transpermethrin in rat and human plasma, rat and human liver microsomes and rat and human liver cytosol; Final Report. Concept Life Sciences Dundee, Dundee Technopole, Dundee, United Kingdom. Laboratory Project ID: CXR1574-III Transpermethrin Amendment 1, May 31, 2018. MRID 50600306. Unpublished.

SPONSOR: Council for Advancement of Pyrethroid Human Risk Assessment, LLC (CAPHRA), c/o Consumer Specialty Products Association, Inc., 1667 K Street, NW, Suite 300, Washington DC.

EXECUTIVE SUMMARY: In a non-guideline, *in vitro* metabolism study (MRID 50600306), the apparent intrinsic clearance (CL_{int}) of transpermethrin (98.5% a.i.; Lot # G026:89-2) was determined in human liver microsomes, liver cytosol, and plasma, as well as juvenile and adult rat liver microsomes, liver cytosol, and plasma (See Appendix; Source of test systems). The study was performed incubating human as well as juvenile (15-day and 21-day old) and adult (90-day old) Sprague-Dawley rat liver microsomes, liver cytosol, and plasma in the presence and absence of NADPH with 0.1-5.0 µM transpermethrin. Liver microsomes and cytosols were incubated at 0.1 mg/mL; plasma was incubated at a dilution of 1:1000. The 1:1000 dilutions yielded protein concentrations of 0.046, 0.048, and 0.077 mg/mL for 15-day, 21-day, and 90-day old rat plasmas, respectively, and 0.079 mg/mL for human plasma. Reactions were stopped by the addition of bifenthrin (internal standard; 98.1% a.i.; Batch # PL09-0427). Incubations were performed on two separate days using newly prepared transpermethrin solutions. Metabolism in the presence of NADPH reflected both NADPH-dependent cytochrome P450 (CYP) metabolism and NADPH-independent carboxylesterase (CES) metabolism. In contrast, NADPH-free incubations measured only CES metabolism. Using these results, CYP-only metabolism was

estimated by the difference between the two metabolic rates. Because CYP activity is not observed in liver cytosol and plasma, only transpermethrin metabolism by CES enzymes was investigated in these compartments. Transpermethrin metabolism, measured as loss of transpermethrin in the samples, was determined by LC-MS/MS. The Michaelis constant (K_m) and maximum reaction rate (V_{max}) were determined using Michaelis-Menten kinetics and the apparent CL_{int} was calculated using the equation: $CL_{int} = V_{max} \div K_m$.

RESULTS: Transpermethrin was metabolized by both CYP and CES enzymes in rat liver microsomes. Total CL_{int} (CYP and CES enzyme activity) was greatest for the 90-day old microsomes (4.37-5.31 mL/min/mg), decreasing in the 21-day old (3.08-3.21 mL/min/mg) and 15-day old (1.22-2.27 mL/min/mg) microsomes. The proportion metabolized by the CYP enzymes decreased with increasing age. In the 15-day old microsomes, the CL_{int} for the CYP enzymes was 0.31-1.33 mL/min/mg, and the CL_{int} for the CES enzymes was 0.84-0.89 mL/min/mg. In the 21-day old microsomes, the CL_{int} for the CYP enzymes was 1.03-2.23 mL/min/mg, and the CL_{int} for the CES enzymes was 1.56-1.74 mL/min/mg. In the 90-day old microsomes, the CL_{int} for the CYP enzymes was 1.13-1.56 mL/min/mg, and the CL_{int} for the CES enzymes was 3.70-3.96 mL/min/mg (Appendix, Table 1). In human liver microsomes, metabolism was entirely due to CES enzymes (1.57-1.80 mL/min/mg), with no metabolism attributed to the CYP enzymes.

Transpermethrin was also metabolized by CES enzymes in rat and human liver cytosol and rat plasma. Rates of transpermethrin metabolism were greatest in 21-day old rat liver cytosol (3.19-3.23 mL/min/mg), with lower values in the 90-day (2.51-2.84 mL/min/mg) and 15-day old (0.93-1.08 mL/min/mg) rat liver cytosols (Appendix, Table 2). Human liver cytosol (1.09-1.10 mL/min/mg) demonstrated a rate slightly lower than human liver microsomes. Rates of transpermethrin metabolism were greatest in 90-day old rat plasma (67.7-80.6 mL/min/mL), decreasing in 21-day old rat plasma (18.6-20.7 mL/min/mL) and 15-day old rat plasma (6.06-6.59 mL/min/mL). Transpermethrin was not metabolized by human plasma (Appendix, Table 3).

COMMENTS: Transpermethrin was metabolized by both CYP and CES enzymes present in rat liver microsomes but was attributed to CES enzymes in both human liver microsomes and cytosol. Overall transpermethrin metabolism by rat microsomes increased with age. No CES metabolism was noted in human plasma.

APPENDIX:

Source of test systems:

Rat: Liver tissue and plasma from 15-day, 21-day, and 90-day old Sprague-Dawley rats (Charles River Laboratories, Raleigh, NC) were shipped to the performing laboratory. Pooled liver microsomal and cytosolic fractions were prepared for each age group (Study Number CXR1572).

Human: Human liver microsomes (Lot # 38290) and cytosol (Lot # 38290) were purchased from Corning B.V. Life Sciences, Amsterdam, The Netherlands. Human plasma (Lot # BRH1076793) was purchased from Sera Laboratories International, Haywards Heath, West Sussex, UK.

TABLE 1 Transpermethrin Liver Microsomes Summary Kinetics

Species	Experiment Date	Replicates in experiment	NADPH	Vmax nmol/min/mg protein	Km µM	Clint mL/min/mg
Rat 90 day	13-Feb-17	n=3	+	16.2	3.05	5.31
			-	9.01	2.44	3.70
			CYP only	8.52	5.48	1.56
Rat 90 day	15-Feb-17	n=3	+	29.8	6.82	4.37
			-	12.4	3.13	3.96
			CYP only	10.1	8.95	1.13
Rat 21 day	22-Feb-17	n=3	+	5.38	1.67	3.21
			-	4.84	2.79	1.74
			CYP only	1.04	0.467	2.23
Rat 21 day	24-Feb-17	n=3	+	4.65	1.51	3.08
			-	4.98	3.20	1.56
			CYP only	1.43	1.39	1.03
Rat 15 day	16-Feb-17	n=3	+	2.31	1.01	2.27
			-	3.18	3.80	0.836
			CYP only	0.793	0.597	1.33
Rat 15 day	20-Feb-17	n=3	+	3.59	2.95	1.22
			-	2.36	2.66	0.885
			CYP only	1.43	4.67	0.306
Human	28-Feb-17	n=3	+	6.12	3.89	1.57
			-	7.04	3.90	1.80
			CYP only	None present (inferred from data above)		
Human	01-Mar-17	n=3	+	5.44	3.30	1.65
			-	4.46	2.59	1.72
			CYP only	None present (inferred from data above)		

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TABLE 2 Transpermethrin Liver Cytosol Summary Kinetics

Species	Experiment Date	Replicates in experiment	NADPH	Vmax nmol/min/mg protein	Km µM	Clint mL/min/mg
Rat 90 day	10-Mar-17	n=3	-	1.27	0.508	2.51
Rat 90 day	10-Mar-17	n=3	-	1.32	0.466	2.84
Rat 21 day	17-Mar-17	n=3	-	1.92	0.594	3.23
Rat 21 day	17-Mar-17	n=3	-	2.04	0.638	3.19
Rat 15 Day	20-Mar-17	n=3	-	1.51	1.63	0.929
Rat 15 Day	20-Mar-17	n=3	-	1.59	1.47	1.08
Human	27-Mar-17	n=3	-	1.51	1.38	1.10
Human	27-Mar-17	n=3	-	1.67	1.53	1.09

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TABLE 3 Transpermethrin Plasma Summary Kinetics

Species	Experiment Date	Replicates in experiment	NADPH	Vmax nmol/min/mL plasma	Km µM	Clint mL/min/mL
Rat 90 day	14-Mar-17	n=3	-	45.4	0.670	67.7
Rat 90 day	14-Mar-17	n=3	-	44.2	0.548	80.6
Rat 21 day	21-Mar-17	n=3	-	19.3	1.03	18.6
Rat 21 day	21-Mar-17	n=3	-	21.8	1.06	20.7
Rat 15 Day	22-Mar-17	n=3	-	4.94	0.749	6.59
Rat 15 Day	22-Mar-17	n=3	-	3.76	0.621	6.06
Human	07-Mar-17	n=3	-	No metabolism detected		
Human	07-Mar-17	n=3	-	No metabolism detected		

C_{Lint} was not determined in human plasma as K_m values were above the range of the assay.

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